Example 15: Identification of Compounds that Modulate Kinase Signaling Cascade Associated with Atherosclerosis

[0672] Many animal models for atherosclerosis have been developed and characterized. For a review of animal models of atherosclerosis, restenosis and endovascular graft research, see e.g., Narayanaswamy et al., JVIR, vol. 11(1): 5-17 (2000), which is hereby incorporated by reference in its entirety. Atherosclerosis is induced in a suitable animal model using a high fat/high cholesterol (HFHC) diet. The test animal is an animal that contains cholesterol ester transferase, such as the rabbit or the swine. The HFHC diet is produced, e.g., using commercial chow supplemented with fat. Cholesterol intake is between 0.5-2.0% of the diet. A test group of animals, e.g., rabbits or swine, receives a compound of the invention. The effect of the test compound is compared to the effects of atherosclerosis in the untreated, control group of animals. Effects that are compared include, for example, the degree of plaque formation, the number and/or frequency of myocardial infarctions observed in each group of animals, and the extent of tissue damage secondary to myocardial infarction exhibited in coronary tissue.

[0673] Myocardial infarction is studied using a variety of animal models such as rats and mice. The majority of myocardial infarctions result from acute transbotic occlusion of pre-existing atherosclerotic plaques of coronary arteries, which is mimicked in animal models by ligation of the left coronary artery in e.g., rats and mice. Myocardial infarction induces global changes in the ventricular architecture, a process called ventricular remodeling. The infarcted heart progressively dilates and accelerates the deterioration of ventricular dysfunction that eventually results in heart failure.

[0674] Myocardial ischemia is induced in test and control groups of animals, e.g., mice or rats, by ligating the left anterior descending coronary artery. The affected heart tissue is contacted with a compound of the invention, for example, by intraperitoneal (i.p.) injections, after the induction of ischemia. High resolution magnetic resonance imaging (MRI), dry weight measurements, infarct size, heart volume, and area at risk are determined 24 hours postoperatively. Survival rates and echocardiography are determined at various times postoperatively in the rats receiving injections of the compound of the invention. Other effects of the test compound are compared to the control group of rats. For example, changes in left ventricular geometry and function are characterized using echocardiography to compare end-diastolic diameters, relative wall thickness, and the percentage of fractional shortening. In excised hearts, the infarct size calculated and expressed as a percentage of left ventricular surface area.

Example 16: Identification of Compounds that Modulate Kinase Signaling Cascade Associated with Neuropathic Pain

[0675] Many animal models for neuropathic pain, such as chronic neuropathic pain, have been developed and characterized, see e.g., Bennett & Xie, Pain, vol. 33, 87-107 (1988); Seltzer et al., Pain, vol. 43, 205-18 (1990); Kim & Chung, Pain, vol. 50, 355-63 (1992); Malmberg & Basbaum, Pain, vol. 76, 215-22 (1998); Sung et al., Neurosci Lett., vol. 246, 117-9 (1998); Lee et al., Neuroreport, vol. 11, 657-61 (2000); Decosterd & Woolf, Pain, vol. 87, 149-58 (2000); Vadakkan et al., J Pain, vol. 6, 747-56 (2005), each of which is hereby incorporated by reference in its entirety. For a review of animal models used for neuropathic pain, see e.g., Eaton, J. Rehabilitation Research and Development, vol. 40(4 Supplement):41-54 (2003), the contents of which are hereby incorporated by reference in their entirety.

[0676] Compounds that modulate neuropathic pain are identified using any of the art-recognized models for neuropathic pain. For example, the models for neuropathic pain generally involve injury to the sciatic nerve, although the method used to induce injury varies. For example, the sciatic nerve is injured due to partial constriction, complete transection, freezing of the nerve, and metabolic, chemical, or immune insults to the nerve. Animals with these types of nerve injury have been shown to develop abnormal pain sensations similar to those reported by neuropathic pain patients. In the studies described herein, the sciatic nerve of test and control groups of subjects, such as mice, are injured. In the test group, subjects are administered a compound of the invention at a variety of times prior to, during and after injury to the sciatic nerve. The effects of the compound on the test group are compared to the effects observed in the control group, e.g., through physical observation and examination of the subjects. For example, in mice, the subject's hindpaw is used to test the response to non-noxious stimuli, such as tactile stimulation, or to test the subject's response to stimuli that would be noxious in the course of ordinary events, for example, radiant heat delivered to the hindpaw. Evidence of allodynia, a condition in which ordinarily nonpainful stimuli evoke pain, or a hyperalgesia, the excessive sensitiveness or sensibility to pain, in the test subjects indicates that test compound is not effectively modulating neuropathic pain in the test subjects.

Example 17: Identification of Compounds that Modulate Kinase Signaling Cascade Associated with Hepatitis B

[0677] Many animal models for hepatitis B have been developed and characterized. For a review of animal models of hepatitis B, see e.g., Guha et al., Lab Animal, vol. 33(7):37-46 (2004), which is hereby incorporated by reference in its entirety. Suitable animal models include, for example, the chimpanzee, tree shrews (non-rodent small animals that are phylogenetically close to primates, see Walter et al., Hepatology, vol. 24(1): 1-5 (1996), which is hereby incorporated by reference in its entirety), and surrogate models such as the woodchuck, duck and ground squirrel. (See e.g., Tennant and Gerin, ILAR Journal, vol. 42(2):89-102 (2001), which is hereby incorporated by reference in its entirety).

[0678] For example, primary hepatocytes are isolated from livers of the tree shrew species tupaia belangeri and are infected with HB V. In vitro infection results in viral DNA and RNA synthesis in hepatocytes and secretion hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) into culture medium. Tupaias can also be infected with HBV in vivo, resulting in viral DNA replication and gene expression in tupaia livers. Similar to acute, self-limited hepatitis B in humans HBsAg is rapidly cleared from serum, followed by seroconversion to anti-HBe and anti-HBs.

[0679] Compounds that modulate hepatitis B are identified using any of the art-recognized models for hepatitis B. In the studies described herein, test and control groups of animals, e.g., chimpanzees or tree shrews, are infected with HBV. In